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PASTEURELLA PESTIS: ROLE OF PESTICIN I
AND IRON IN EXPERIMENTAL PLAGUE

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ROLE OF PESTICIN I AND IRON IN EXPERIMENTAL PLAGUE,

10 by Robert R. Brubaker,
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ABSTRACT

Loss of the genetic determinant for pesticin I in Pasteurella pestis results in concomitant loss of the plague coagulase and fibrinolytic factor. The LD₅₀ for mice of an isolate lacking only these activities is increased by factors of about 10¹, 10⁴, and 10⁷ cells when administered by the intravenous, intraperitoneal, and subcutaneous routes, respectively. Virulence of this isolate can be enhanced in mice treated with 40 µg of ferrous iron. This response resembles that of Pasteurella pseudotuberculosis, a closely related species that normally lacks pesticin I.

PASTEURELLA PESTIS:
ROLE OF PESTICIN I AND IRON IN EXPERIMENTAL PLAGUE

Burrows and co-workers^{1,2} have described four properties that are essential for full virulence in Pasteurella pestis, the causative agent of bubonic plague. These properties are the genetic potentials that permit synthesis of purines (Pu^+), virulence antigens (VW^+), and capsular antigen (Fl^+), and permit formation of pigmented colonies on synthetic medium (P^+). The median lethal dose (LD_{50}) in mice and guinea pigs of mutants lacking each of these determinants is shown in Table 1. Production of Fl is not essential for virulence in the mouse and strains that are Pu^+ , VW^+ , and P^+ can be restored to full virulence in this animal by injection of Fe^{++} .³

TABLE 1. INCREASE IN LD_{50} (INTRAPERITONEAL INJECTION) AFTER LOSS OF THE ESTABLISHED VIRULENCE DETERMINANTS OF PASTEURELLA PESTIS¹

Virulence Determinant				LD_{50}	
Pu	VW	Fl	P	Mouse	Guinea Pig
+	+	+	+	<10	<10
0	+	+	+	>10 ⁸	>10 ⁹
+	0	+	+	>10 ⁸	>10 ⁹
+	+	0	+	<10	~10 ⁴
+	+	+	0	>10 ⁸	>10 ⁹

Wild-type strains of P. pestis produce at least two bacteriocin-like substances. The first, termed pesticin I (PI), prevents growth of certain strains of Pasteurella pseudotuberculosis and PI-deficient mutants of P. pestis that remain P^+ .⁴⁻⁶ Pesticin II (PII) is a second bacteriocin-like substance produced by both P. pestis and P. pseudotuberculosis. It is active against many strains of P. pestis that lack PI.⁶

We have shown that an absolute correlation exists between production of PI and expression of the plague coagulase (C) and fibrinolytic factor (F). This finding suggests, but does not prove, that PI, C, and F reside on a hereditary unit distinct from the bacterial chromosome.⁷ Possible factors associated with production of or sensitivity to PII have not yet been investigated. The antibacterial activity of PI, but not PII, is strongly inhibited by ferric ions⁶ and the virulence of wild-type *P. pseudotuberculosis*, like that of F⁻ strains of *P. pestis*, is strongly enhanced in mice treated with Fe⁺⁺.¹

The role of PI, C, and F in the pathogenic process has not yet been determined because of difficulties in obtaining PI-deficient mutants that remain Pu⁺, VW⁺, and F⁺. However, Burrows¹ found that a PI-negative strain of genotype Pu⁺, VW⁺, Fl⁺, and F⁻ was virulent in mice receiving Fe⁺⁺. Thus, he suggested that either PI was not essential for virulence or that Fe⁺⁺ fulfilled a dual requirement by replacing the products of both F⁺ and PI⁺.

Against this background, a report by Eisler⁸ describing certain partially virulent strains of *P. pestis* as lacking C was of interest and Dr. Eisler kindly made these strains available. As expected, all strains lacked detectable PI and F; however, only strain G-32 proved to be both VW⁺ and F⁺. The genotype of strain G-32 is compared in Table 2 with those of wild-type *P. pestis* (strain Alexander) and *P. pseudotuberculosis* (strain PBI/+) with respect to established virulence determinants and other distinguishing properties. Table 3 shows the LD₅₀ of these strains by three routes of injection in normal Fort Detrick mice and in those receiving individual intraperitoneal injections of 40 µg of Fe⁺⁺.

TABLE 2. SOME PROPERTIES OF *P. PESTIS* STRAIN ALEXANDER (WILD TYPE), *P. PESTIS* STRAIN G-32, AND *P. PSEUDOTUBERCULOSIS* STRAIN PBI/+ (WILD TYPE)

Strain	Pu	VW	Fl	P	PI	C	F	T ^a /	4 ^a /	U ^a /
Alexander	+ ^b /	+	+	+	+	+	+	+	+	-
G-32	+	+	+	+	-	-	-	+	+	-
PBI/+	+	+	-	+	-	-	-	-	+	+

a. T = murine toxin, 4 = antigen 4, and U = urease; the significance of murine toxin and antigen 4 are discussed by Burrows.¹

b. Presence: +; absence: -.

TABLE 3. RESULTS OF LD₅₀ DETERMINATIONS FOR P. PESTIS STRAIN
ALEXANDER, P. PESTIS STRAIN G-32,
AND P. PSEUDOTUBERCULOSIS STRAIN PBI/+ IN NORMAL AND TREATED MICE^a/

Strain	No Added Fe ⁺⁺	Plus 40 µg Fe ⁺⁺
<u>Intravenous Injection</u>		
Alexander	8.1x10 ⁰ (4.9x10 ⁰ -1.5x10 ¹)	9.8x10 ⁰ (5.6x10 ⁰ -1.5x10 ¹)
G-32	7.1x10 ¹ (4.0x10 ¹ -1.4x10 ²)	2.3x10 ¹ (1.3x10 ¹ -4.2x10 ¹)
PBI/+	3.9x10 ¹ (2.2x10 ¹ -6.6x10 ¹)	3.1x10 ¹ (1.6x10 ¹ -5.7x10 ¹)
<u>Intraperitoneal Injection</u>		
Alexander	9.9x10 ⁰ (5.7x10 ⁰ -1.6x10 ¹)	9.8x10 ⁰ (5.6x10 ⁰ -1.5x10 ¹)
G-32	3.8x10 ⁵ (1.8x10 ⁵ -8.1x10 ⁵)	1.4x10 ¹ (7.3x10 ⁰ -2.5x10 ¹)
PBI/+	2.9x10 ⁴ (1.8x10 ⁴ -4.7x10 ⁴)	2.0x10 ² (1.1x10 ² -3.5x10 ²)
<u>Subcutaneous Injection</u>		
Alexander	6.1x10 ⁰ (4.0x10 ⁰ -1.0x10 ¹)	5.9x10 ⁰ (3.7x10 ⁰ -9.3x10 ¹)
G-32	>5.0x10 ⁸	3.2x10 ⁸ (1.7x10 ⁸ -5.9x10 ⁸)
PBI/+	1.1x10 ⁴ (5.5x10 ³ -1.7x10 ⁵)	1.4x10 ² (7.3x10 ¹ -2.5x10 ²)

a. LD₅₀ is expressed as number of cells and values in parentheses represent 95% confidence limits as calculated by the method of Goldberg, et al.⁹; immediately before challenge Fe Cl₂ was injected intraperitoneally in 0.1 ml peanut-oil suspension.

The LD₅₀ for strain Alexander was less than 10 cells in treated and normal mice by all methods of infection. The intravenous LD₅₀ of strains G-32 and PBI/+ was only slightly higher. These two strains showed reduced virulence after intraperitoneal injection in normal mice; high virulence could be restored by treatment with Fe⁺⁺. Strain G-32 was avirulent when injected subcutaneously into both treated and control animals. Strain PBI/+ responded differently by this route, being partially and almost fully virulent in normal and treated mice, respectively.

These results demonstrate that PI is associated with high virulence in normal mice infected by the intraperitoneal or subcutaneous, but not intravenous route, and that Fe^{++} does indeed fulfill a dual role in restoring virulence to mutants lacking P as well as PI. The PI-deficient strains possessed the potential for high virulence, provided that physical barriers of the host were by-passed by intravenous injection. Evidently, PI is associated with the normal ability to overcome these barriers, that is, the property of invasiveness. Further interpretation as to which gene products are critical and the role of Fe^{++} must be made with caution.

In general, bacteriocins are products of non-chromosomal genes that exist on cytoplasmic replicating units termed plasmids.¹⁰ Episomes are a related class of determinants, governing the expression of temperate bacteriophages, that are capable of integration on the chromosome or autonomous existence in the cytoplasm.¹¹ Although extra-chromosomal genes are seldom essential for bacterial growth, they may facilitate survival in special environments such as those encountered by pathogens. For example, certain bacteriophage genomes confer on their bacterial hosts the ability to produce toxins.¹²⁻¹⁵ Lysogeny can also result in antigenic changes that may facilitate survival of bacteria in otherwise immune mammalian hosts.^{16,17} In these cases, enhanced virulence is physiologically associated with toxin molecules or altered antigenic sites rather than with vegetative bacteriophages. By analogy, the products of C and F rather than of PI would be associated with invasiveness in *P. pestis*. Nevertheless, Burrows* suggested that PI may possess siderophilin activity for *P. pestis* but act as a sideromycin for sensitive strains of *P. pseudotuberculosis*. This suggestion is attractive because *P. pestis* can grow slowly in nonhemolyzed serum but *P. pseudotuberculosis* does not. Both species grow rapidly after saturation of serum transferrin with Fe^{++} .¹⁸ These results would be expected if *P. pestis* possesses a unique ability to obtain iron in vivo by chelation. However, the results in Table 3 indicate that loss or normal absence of this proposed activity is of little consequence to bacteria injected intravenously. An alternative suggestion is that strains lacking PI are sensitive to a normal antibacterial component of serum that is inactivated by Fe^{++} . An anti-respiratory 7S globulin that fits this description has been described.¹⁹

The major physiological determinant of virulence in *P. pestis* appears to be its ability to survive and multiply within phagocytes, especially within fixed macrophages of the reticuloendothelial system.²⁰⁻²³ Intracellular residence affords protection against normal antibacterial components of serum. The PI-deficient cell may fail to obtain a favored anatomical site in the mouse, suitable for intracellular growth, unless it is administered directly into the vascular system. The ability of wild-type *P. pestis* to obtain such sites after intraperitoneal or subcutaneous injection may be influenced by C and F rather than by PI. To resolve the roles of PI, C, and F completely, it may prove necessary to isolate PI⁻ strains that remain C⁺ and F⁺, or vice versa, by introduction of suitable point mutations on the PI determinant.

* T.W. Burrows, personal communication.

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